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Research and Development Report

THE EFFECTS OF MICROBIAL BIOFILMS ON ORGANOTIN RELEASE BY AN ANTIFOULING PAINT

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Abbreviations

ml	millimeters
ppt	parts per thousand
mm	millimeters
ASTM	American Standard Testing Methods
C	Centigrade
hr	hours
ug_2	micrograms
cm^2	centimeters squared
TBT	tributyltin
DBT	dibutyltin
GC	Gas Chromatography

Abstract

The effect of microbial films on the release rate and speciation of tributyltin toxin from an organotin paint was determined. Test panels were coated with organotin copolymer ablative antifouling paint. The release rates of the panels were measured before and after biofilms were formed, and after they were mechanically removed. Results show that the bacterial films decreased the release rate but did not degrade the tributyltin. Algal films showed an increase in the release rate with an accompanying dedragation of the tributyltin to di- and monobutyltin. For all panels the release rates significantly increased after the biofilms were removed.

Administrative Information

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Introduction

Organotin-containing antifouling paints are very effective against marine hard fouling[1]. However, microbial biofilms have been found on such coatings during service. The environmental assessment of the implementation of organotin-containing antifouling requires knowledge of the release rate of this toxin into the environment. Therefore, any differences in organotin release rates or chemical speciation due to the formation of biofilms on the coating should be taken into account when environmental impacts are assessed.

Measurements of release rates made on painted surfaces which have been exposed to natural waters, and so support a microbial biofilm, would have to be made first with the biofilm

in place and then after the biofilm had been scraped from the surface. The physical removal of the biofilm would disturb the painted surface and not give a true measurement of the release rate of a biofilm free coating. The significant requirement for this work was to maintain a non-biofilmed surface long enough to establish a baseline release rate for the unfilmed paint. This work consisted of preparing panels coated with an organotin antifouling paint whose release rate would be measured before and after biofilms had been formed on the coated panels. Determining the release rate after the biofilm was removed would allow comparisons with data taken in field experiments.

Methods

The experimental panels consisted of 3 by 5 inch steel Q-panels. Panels were cleaned with xylene and then coated with 3 mils of F-150 epoxy primer Mil 24441, a 1 mil tack coat of the primer, followed by a 3 mil coating of organotin copolymer ablative antifouling paint. Panel immersion chambers consisted of 940 ml glass jars which contained 850 ml ASTM class 2 seawater at 34 ppt salinity. Polycarbonate hangers were used to suspend the panels in the seawater. The glass jars were placed in a 22^{o}C temperature control system containing a reciprocal shaker with a 4 mm stroke at 72 strokes per minute to thoroughly mix the seawater around the panel.

Stock ASTM seawater was prepared by dissolving the salt mix in distilled water to a salinity of approximately 70 ppt and stored in covered glass aquariums with constant aeration.

Microorganism growth was prevented by passing the seawater through a ultra-violet aquarium sterilizer at a rate of 100 ml/minutes. Prior to use, working seawater solutions were diluted to 34 ppt with distilled water. Periodic checks of the microorganism population in the aquarium holding tanks were conducted.

After the panels were placed in the test jars the 850 ml of seawater was changed twice weekly to prevent build-up of organotin concentrations. Water from these test jars (200 ml) were used for analysis of the amount and speciation of the released organotin. The tin extraction method consisted of an initial extraction with 10 ml methylene chloride, 10 minutes of vigorous shaking, 10 minutes phase separation, followed by recovering the methylene chloride from the water sample[2]. These steps were then repeated with a fresh portion of methylene chloride and the two 10 ml aliquots combined. A modification to Olsons method involved the omission of the sodium borohydrate during the initial extraction process, Matthias (personal communication), since our extracted samples were to be stored (0°C) for 2 to 3 months prior to analysis, and sodium borhydrate during storage could have resulted in degradation of the sample. The sodium borohydrate was added immediately before separation of mono-, di- and tributyltin by gas chromatography and measurement of the tin concentrations with flame ionization detection.

Midway into the experiments temperature control in the laboratory failed leading to widely varying release rates of organotin. The test jars and shaking device were then placed

in a temperature controlled chamber at 22°C, and a stable release rate of organotin documented.

After a stable release rate was obtained panels were inoculated with bacterial and algal cultures consisting of organisms cultured from the hull of a ship which is coated with an organotin antifouling paint. Bacteria were cultured in Difco marine broth (2261), while the algae were cultured in formula F2, both standard culture media. Biofilms were formed on the panels by inoculations, each followed by a change of seawater after a 24 hr filming period. These films were allowed to remain on panels A, B, C, and F for 38 days, with samples for analysis being taken twice a week when the seawater was changed. After 38 days the thickness of the biofilm was measured on the panels using a light section microscope, and the biofilms removed and samples taken for identification. Panels were then placed back into the test jars to determine the release rates after the films were removed. On other panels, D, E, and F, biofilms were not removed after the 38 days, but remained on the panels for the 110 day duration of the experiments.

Results

Stable release rates for the unfilmed panels were established at 0.11 to 0.16 ug/cm2/day TBT (avg. TBT 0.135± 0.06 ug/cm2/day), with a release of dibutyltin of <0.001 ug/cm2/day (Table 1, Figure 1). Panels A-E were inoculated with the bacteria cultures and panels F and G with the algal culture.

Table 1. Average tributyl and dibutyltin release rate for panels without biofilms, after biofilms developed, and after these films had been removed.

		Release Rate in μ g/cm2/day					
Panel Number	Film Type	Without Biofilm TBT[3]	DBT[4]	With Biofilm TBT	DBT	Biofilm Removed TBT	DBT	Biofilm Thickness
A	Bacteria	.16±.02	<.001	.04±.004		<.001	<.001	8.5×10^{-4}
B	Bacteria	.14±.02	<.001	.06±.11		<.001	.004±.002	5.4×10^{-4}
C	Bacteria	.12±.02	<.001	.04±.01		<.001	.005±.002	9.2×10^{-4}
D	Bacteria	.20±.02	<.001	.11±.ID[1]		<.001	ND[2]	ND[2]
E	Bacteria	.18±.02	<.001	.09±.ID[1]		<.001	ND[2]	ND[2]
F	Agal	.11±.02	<.001	.16±.13		.02±.01	.03±.02	6.7×10^{-4}
G	Agal	.17±.04	<.001	.75±.24		.06±ID[1]	ND[2]	ND[2]

[1] Insufficient data

[2] No data

[3] TBT = Tributyltin

[4] DBT = Dibutyltin

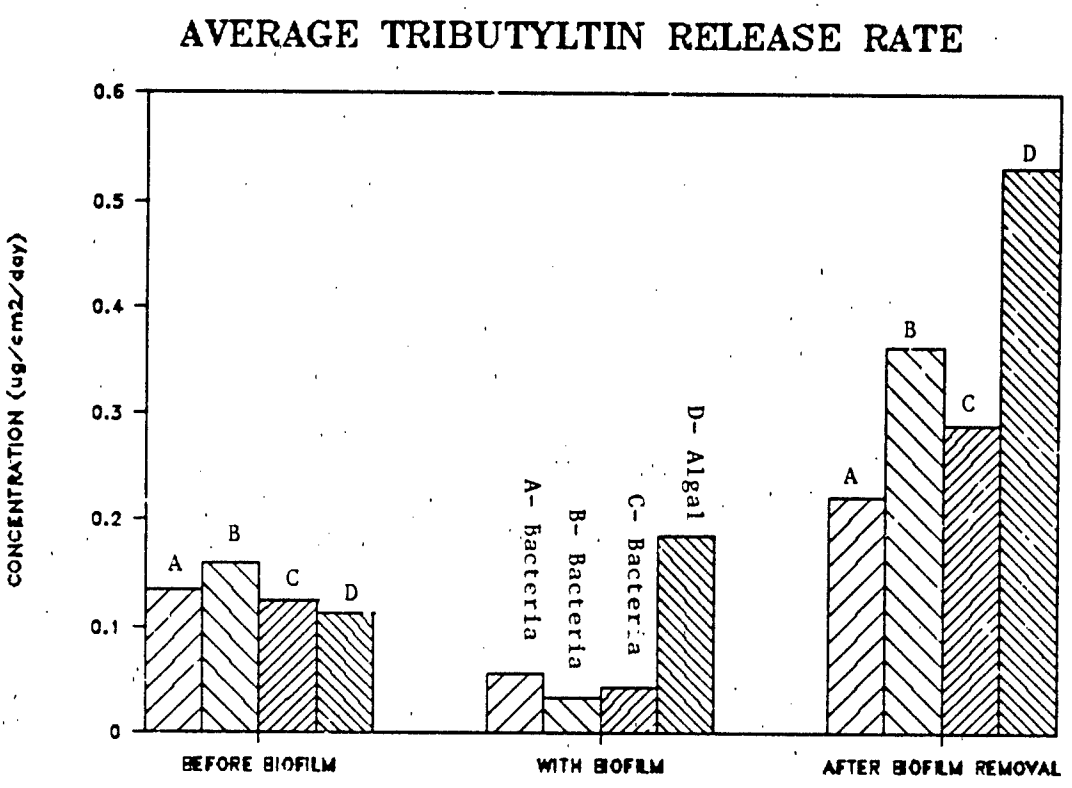

Figure 1. Average tributyltin release rates for 4 test panels before biofilms had formed, after biofilms formed, and after biofilm removal.

After the bacterial films formed on panels, the release rate decreased 60% to an average of 0.07+0.03 ug/cm2/day TBT. Release rates remained at this level for the 38 days the bacterial films were on panels A, B and C. During this period the dibutyltin release rates remained at <0.001 ug/cm2/day (Figure 2). On panels D and E, with the bacterial films not removed, the release rates remained lower than the initial rates for the 110 day remainder of the experiments.

In contrast algal films on panels F and G caused an increase in the release rate to an average of 0.46±0.42 ug/cm2/day TBT (Table 1, Figure 1). Also, the dibutyltin concentrations significantly increased from <0.001 up to 0.02 - 0.06 ug/cm2/day DBT (Figure 3), with a few measurements showing low amounts of monobutyltin. In addition, there were many other chromatograph peaks which are unidentified, but demonstrate the breakdown of the organotin. These substances may be other degradation products, or reaction products of tributyltin metabolites, such as methyl-tin compounds. Identification of these substances will require additional effort. The dominant algal form on these samples was the blue-green alga Anacystis montana identified by Dr. Phillip Sez, Georgetown University.

After the biofilms (bacteria and algal) were removed, release rates increased approximately 2 to 5 times the original stable rate (Table 1, Figure 1).

During GC analysis a very large peak occurred after the tributyltin peak for all paint extracts analyzed (Figure 4). This peak prevented detection of tetrabutyltin, or clear

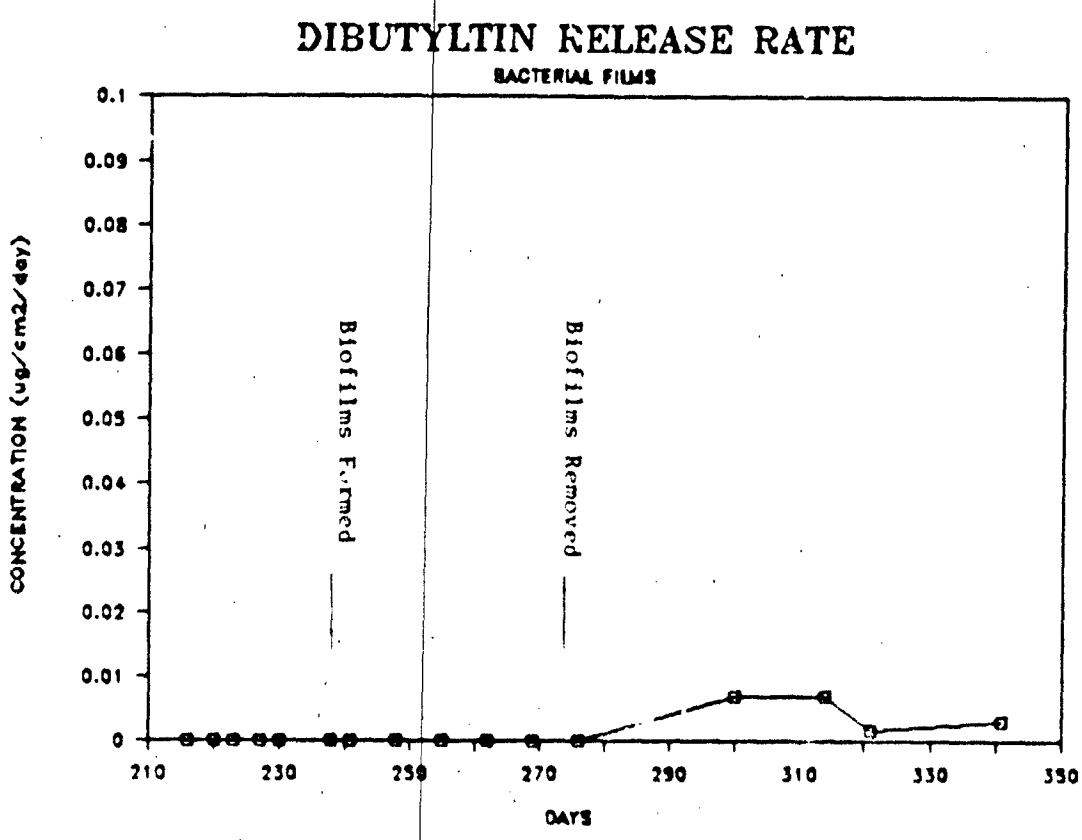

Figure 2. Dibutyltin release rate for all panels tested with bacterial films.

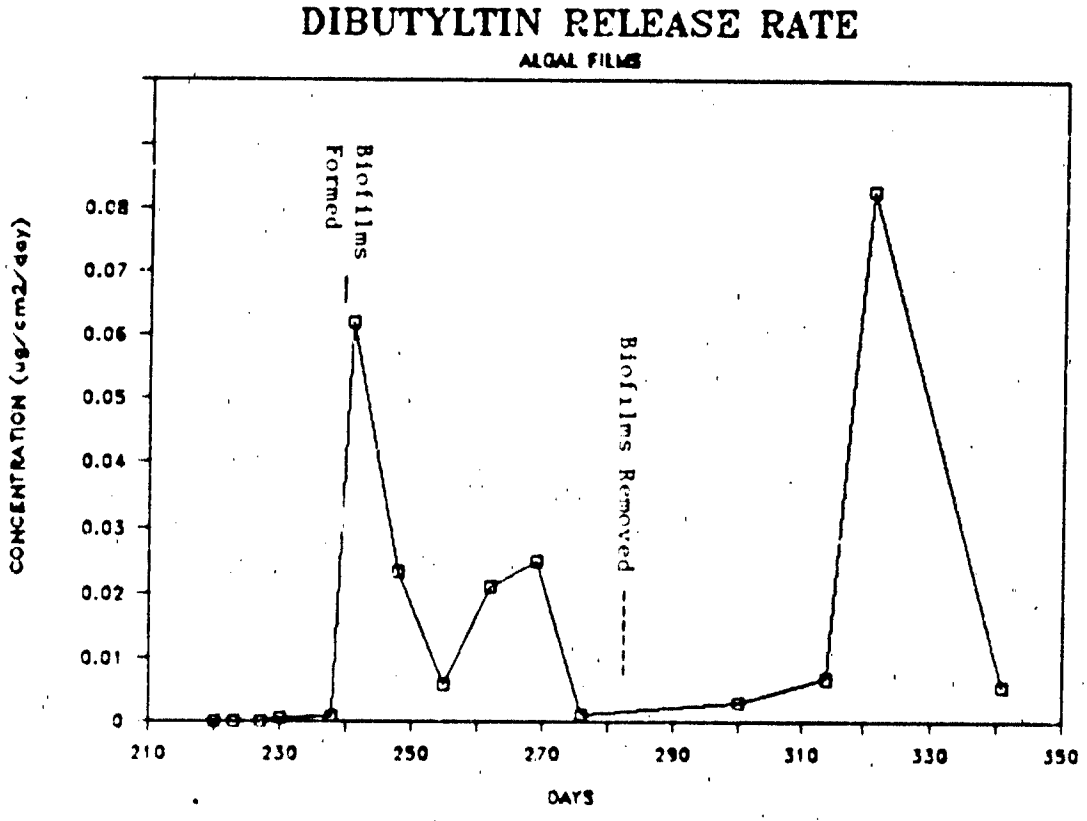

Figure 3. Dibutyltin release rate for all panels tested with algal films.

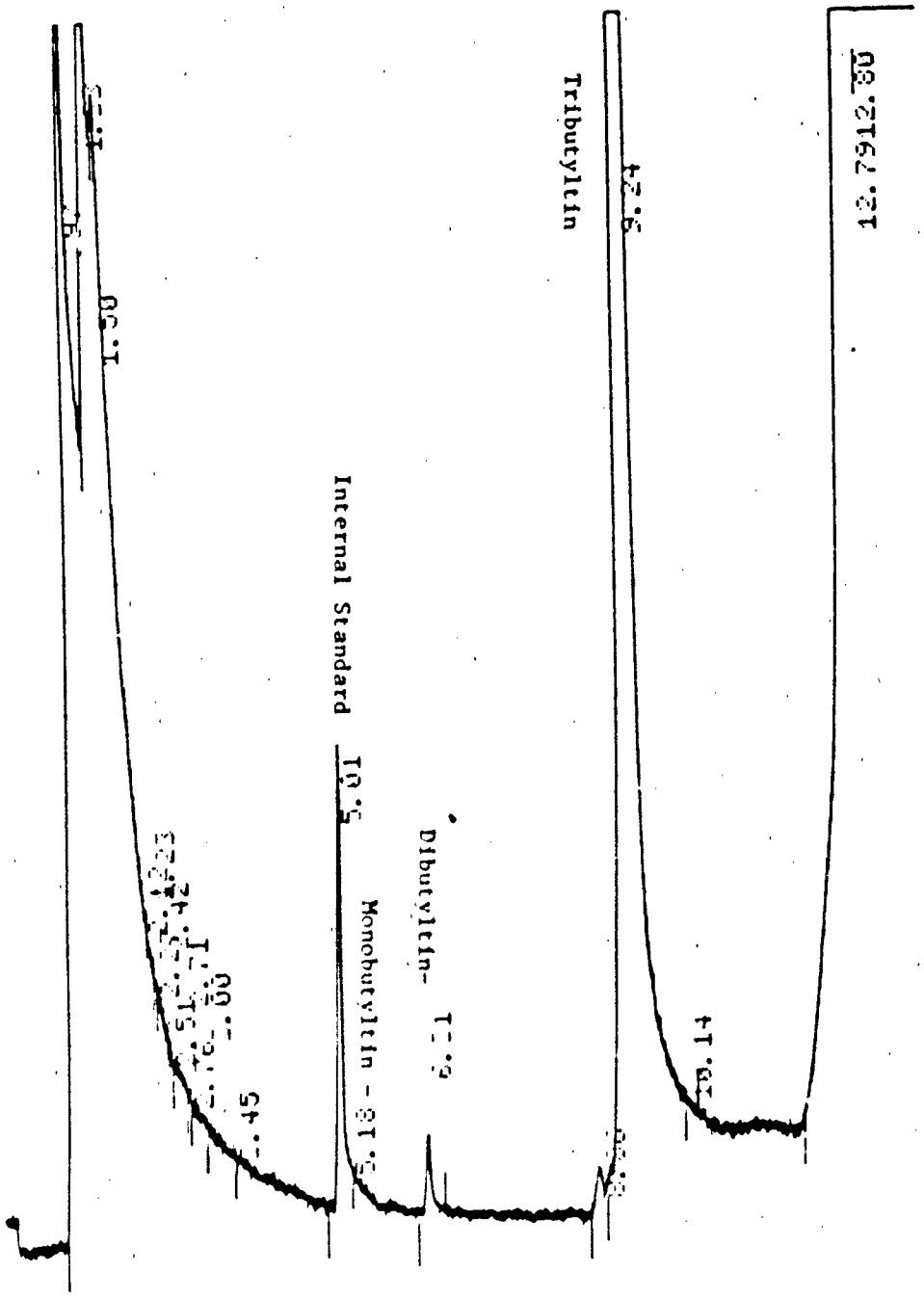

Figure 4. Original gas chromatographfor tin speciation analysis.

resolution of any peaks at longer retention times. Since the detector is biased toward tin detection, it is possible that this signal represents a significant amount of tin-containing material. However, this is not yet been demonstrated definitely, and further studies directed towards elucidation of this point will be required before any clear identification of this material will be achieved.

The observable thickness of the biofilms measured with a light section microscope confirms that biofilms had developed on the panels (Table 1). Comparisons can be made to biofilm thickness measurements made in the natural environment.

Discussion

These results indicate that biofilms have the ability to alter release rates from the paint coating. The bacterial and algal films developed on these samples had quite different effects. Bacterial films decreased the amounts being released. This reduction could be due to an interaction of released material with the extracellular materials produced by the bacteria. Alternatively, diffusion could be significantly reduced in the biofilm, or the bacteria could create an environment which inhibits the release of the organotin from the paint. These effects could be important in designing antifouling paints that would have very low release rates yet still be effective antifoulants because the toxin would be present in the biofilm for any later arriving macrofoulers.

The opposite effect is seen with the algal films, with increased release rates and concurrent breakdown of the tributyltin. This may also be due to the local environment created by the algal metabolites. The nature of the metabolites or their influence on the local water chemistry in the biofilm (such as pH) facilitates the increased release of the toxin from the paint and its passage through the biofilm. It is not clear at this point what the mechanisms are for the breakdown of the tributyltin, however, this type of biodegeration has been reported by other workers[3].

Effects of algal films are considered important to the environmental impacts of tributyltin. Algae can be a highly efficient detoxifying agent in the aquatic environment, especially at installations specially designed to capitalize on this property. Algae can be grown in ponds receiving effluents from a number of processes. Both dispersed cell populations and attached forms, which can be handled on solid surfaces, are well known, with great versatility in the engineering community. Their use has been evaluated in European power stations for this purpose, with highly encouraging results of more than 90% conversion to inorganic tin in short times[4]. However, we may expect that the implications of biotransformation of even inorganic tin to methyl tin or similar materials in estuarine sediments will have to be considered.

The availability of algal detoxification is of great significance for biotechnological processing of organotins. The metabolic processes of naturally occurring bacteria have

not been found suitable for organotin detoxification, however the detoxifying capability of naturally occurring algae allows an immediate biotechnological approach without relying on the development of genetically engineered bacterial strains.

The increase in the release rate after the biofilms were removed is likely due to increasing the paint surface area from the scraping process. This can be a very important consideration for other field and laboratory work which would involve biofilm removal prior to release rate measurement.

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